A Comparative Study of Bile Acid Metabolism in the Rat, Mouse, Hamster, and Gerbil* (33722)

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The relationship between bile acid and tissue sterol metabolism has been well documented (1). While bile acid metabolism has been extensively investigated in the rat, dog, rabbit, and human (1), little information is available in the cases of the mouse, gerbil, and hamster. Moreover, since these rodents differ widely in their tendencies to accumulate tissue sterols when fed diets supplemented with cholesterol, a comparative study of bile acid metabolism in these species and in the rat should be of interest. We therefore investigated: (a) the qualitative and quantitative aspects of bile acid metabolism in the rat, mouse, gerbil, and hamster; (b) the applicability of the methods of Strand (2) and of Linstedt and Norman (3) to the determination of bile acid synthesis rates in the mouse, hamster, and gerbil; and (c) the possible existence of a correlation between the rate of conversion of tissue sterols to bile acids and the ease of accumulation of tissue sterols in the various animals.

Methods and Procedures. Animals. The following animals were used in the experiments: female albino mice of the Webster strain, weighing 22–25 g; female Sprague-Dawley albino rats, weighing 250–275 g; female Golden hamsters, weighing 90–110 g; and female Mongolian gerbils, weighing 60–90 g. These animals were maintained *ad libitum* on a ground commercial rat diet containing (%): protein 24.27; fat, 4.15; fiber, 4.86; carbohydrate, 56.23; and ash, 7.78. Food consumption, weight changes, and general health were monitored for a 1-month observation period. The average daily food consumptions per animal were (g): mouse, 4; rat, 12; hamster, 6; and gerbil, 5.

Purification of cholic acid-24-^{14}C and chenodeoxycholic acid-24-^{14}C. Glass plates (20 × 20 cm) were coated with 250 μ layers of silica gel G. One of the bile acids (in amounts up to 5 mg) was applied uniformly along a line 1 cm above the bottom of the plates. The plates were developed using appropriate solvent systems (4), dried, and then sprayed with water to locate the areas containing the bile acids. After marking these areas, the plates were dried, the marked areas were removed by scraping, and the purified bile acids were eluted with ethanol. Small aliquots of the acids were checked for purity and identity by thin-layer chromatography using spray reagents which yield specific colors with various bile acids (5).

Expt. A. Bile acid-24-^{14}C side-chain metabolism. Two μCi of either chenodeoxycholic acid-24-^{14}C or cholic acid-24-^{14}C was injected intraperitoneally in 4 rodents of each species. The animals were placed in individual glass metabolism cages, and the expired CO_2 was collected in 40% ethanolic ethanolamine for 40 hr. Aliquots of the resulting solution were added to 15 ml of phosphor solution (5 g of 2,5,-diphenyloxazole and 0.3 g of 1,4-bis [2-(4-methyl-5-phenyloxazolyl)]-benzene dissolved in 1 liter of toluene and 133 ml of ethanol), and the activity determined in a Tri-Carb scintillation spectrometer. An automatic external standard was used to correct for quenching in all cases.

Expt. B. Urinary bile acid-24-^{14}C excretion. Six animals of each species except mice received intraperitoneal injections of cholic acid-24-^{14}C or chenodeoxycholic acid-24-^{14}C. Urine was collected for 9 days.

Urinary bile acid-^{14}C activity was determined by counting aliquots of 24-hr urine collections added to 15 ml of phosphor solution [7 g of 2,5-diphenyloxazole; 0.3 g of 1,5-bis2-(4-methyl-5-phenyloxazolyl)-benzene and 100 g of naphthalene dissolved in 1 liter of dioxane].

Expt. C. Distribution of bile acid-24-^{14}C in

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the various pool sites in rats, mice, hamsters, and gerbils. Four animals of each species received 2 μCi intraperitoneal injections of either chenodeoxycholic acid-24-^{14}C or cholic acid-24-^{14}C. Forty hr later the animals were killed, and small intestines, cecums, gall bladders (all plus contents) and livers were removed. Small intestines and gall bladders were combined. After lyophylizing, the bile acid fraction was isolated from each tissue (4, 6) and ^{14}C activity was determined in aliquots of the fraction as outlined in Expt. A.

Expt. D. Bile acid half-lives, pool sizes and spectra of various rodents. Groups of 8 animals of each species received intraperitoneal tracer doses (rats, 5 μCi; mice, 1 μCi; hamsters and gerbils, 2 μCi) of cholic acid-24-^{14}C (4.03 mCi/mmole); similar groups received chenodeoxycholic acid-24-^{14}C (1 mCi/mmole) at the same dose level. After injection, each animal was placed in an individual metabolism cage, and feces were collected daily for 9 days.

The animals were then killed and the ceca, small intestines, large intestines, gall bladders (all plus contents) and livers were removed. Gall bladders and small intestines were combined. All of the feces and tissue samples were dried by lyophilization.

Two methods were used to prepare the feces and tissue samples for counting bile acid-24-^{14}C activity. Either the samples were exhaustively extracted with boiling ethanol and aliquots of this extract were counted; or they were combusted by the Schoniger oxygen-flask method (7) and $^{14}CO_2$ was collected in 40% ethanolic ethanolamine and counted. Tests on feces and tissue samples from mouse, rat, and gerbil demonstrated good agreement in the results by the two methods for these species. However, extraction could not be used in the case of hamster feces samples because this procedure, which yielded nearly complete recovery of ^{14}C activity in the other 3 species, resulted in a 75% loss of activity in the feces samples from the hamster. Although several solvents and combinations of solvents were investigated no satisfactory extraction procedure was found for these samples. Tissue extraction was satisfactory.

TABLE I. $^{14}CO_2$ Expired Following Cholic Acid-24-^{14}C or Chenodeoxycholic Acid-24-^{14}C Injection in Various Rodents.

	Percentage of ^{14}C activity expired in 40 hr after injection	
Species	Cholic acid	Chenodeoxycholic
Rat	0.22 ± 0.07	0.11 ± 0.03
Mouse	0.26 ± 0.10	0.21 ± 0.15
Hamster	0.55 ± 0.09	0.40 ± 0.23
Gerbil	0.65 ± 0.21	0.46 ± 0.35

The ^{14}C activities of feces and tissue sample extracts or combustion products were determined by scintillation counting as outlined above (Expt. A).

The bile acid half-life was calculated from the counting data according to Linstedt and Norman (3). To determine the bile acid pool size and spectrum, the lyophylized small intestine and gall bladder samples were exhaustively extracted with boiling ethanol. The bile acid-containing fraction was isolated by a modification of the method of Siperstein (4, 6), and the individual bile acids were separated and quantitated by methods developed in our laboratory (4). The total bile acid pool size was calculated according to Strand (2). The identity of the bile acids in the pool was established by comparisons of R_f values with standards, and by color development of thin-layer chromatograms, using special spray reagents which yield definitive colors with various bile acids (5).

Results and Discussion. Expt. A. Side-chain oxidation of cholic and chenodeoxycholic acids in various rodents. Table I shows the percentage of ^{14}C activity recovered in expired CO_2 following intraperitoneal injection of cholic acid-24-^{14}C and chenodeoxycholic acid-24-^{14}C in rats, mice, hamsters, and gerbils. It is obvious that bile acid side-chain degradation is an insignificant metabolic pathway in all of these animals. Bile acid side-chain oxidation has been shown to be almost nil in several species (1). These findings do not exclude the possibility that some types of bacteria may be able to carry

out this reaction and may at times be present in the gastrointestinal tract.

Expt. B. Urinary excretion of bile acid-^{14}C following injection of cholic acid-24-^{14}C. In all cases studied, the amount of bile acid-24-^{14}C excreted via urine was less than 7% of the injected material. The mouse was not investigated.

Expt. C. Distribution of cholic and chenodeoxycholic acid between the bile acid pool sites in mice, rats, hamsters, and gerbils. Preliminary studies showed that in all of the rodents the bulk of bile acids is confined to small intestine, cecum, gall bladder, and liver. A varying amount of bile acid was also found in the large intestine. However, it is assumed, although it remains to be proven, that there is little or no bile acid absorption taking place in this portion of the gastrointestinal tracts of the different species. In order for Strand's method (2), which we used for calculating the cholic and chenodeoxycholic acid content of the various sections of the bile pool, to be valid, the bulk of the bile acid must be present in the small intestine and gall bladder, where little bacterial alteration of the primary bile acids takes place. We therefore investigated the distribution of bile acid-^{14}C activity in the pool sites 40 hr after injection of a tracer dose of cholic acid-24-^{14}C or chenodeoxycholic acid-24-^{14}C in several animals. The results are shown in Table II. While there are differences, it is apparent that in all cases by far the highest percentage of C^{14} activity is found in the small intestine and gall bladder compartment. The distribution of the two acids runs parallel except in the gerbil, where a higher percentage of chenodeoxycholic acid is found in the cecum.

Expt. D. Bile acid pool: sizes, spectra, and half-lives in rats, mice, hamsters, and gerbils. Chromatrograms comparing the bile acid spectra of the pool acids isolated from the small intestine and gall bladders of mice, hamsters, and gerbils, and from the small intestine of rats are shown in Fig. 1. Cholic acid was the chief primary bile acid present in all species. Chenodeoxycholic acid, the other primary bile acid, was present in each case but the ratio of the amounts of cholic to chenodeoxycholic acid differed widely from species to species. Chenodeoxycholic acid was a prominent feature of the rat and hamster bile acid pool spectra, but was present in only trace amounts in the pools of mice and gerbils.

TABLE II. Percentage Distribution of Bile Acid-24-^{14}C in Bile Acid Pool Sites 40 hr After Intraperitoneal Cholic Acid-24-^{14}C or Chenodeoxycholic Acid-24-^{14}C Injection.

Species	Sm. intestine + G. bladder (%)	Cecum (%)	Liver (%)
	Cholic acid-24-^{14}C injected 2 μCi		
Rat	94.3 ± 2.61	4.67 ± 0.21	1.30 ± 1.2
Mouse	92.7 ± 1.25	4.76 ± 0.73	2.54 ± 0.89
Hamster	80.9 ± 1.54	16.6 ± 2.85	2.50 ± 0.21
Gerbil	91.6 ± 3.96	7.27 ± 3.28	1.13 ± 0.54
	Chenodeoxycholic acid-24-^{14}C injected 2 μCi		
Rat	—	—	—
Mouse	93.9 ± 3.43	2.53 ± 0.52	3.59 ± 0.64
Hamster	83.4 ± 2.32	8.70 ± 0.98	7.90 ± 2.10
Gerbil	71.7 ± 0.28	25.7 ± 3.64	2.86 ± 1.23

Since chenodeoxycholic and deoxycholic acids are difficult to distinguish from one another on thin-layer chromatograms, color development was used to confirm the identity of chenodeoxycholic acid. The acid was present in each case; however, the color reagent confirmed the presence of small amounts of deoxycholic acid running slightly faster than chenodeoxycholic acid. Each of the bile acid pools also contained considerable amounts of muricholic acids, which run somewhat faster than cholic acid on the chromatograms. These acids, along with deoxycholic acid, have been shown to be secondary metabolic products of cholic and chenodeoxycholic acids (1).

It is interesting that the bile acid pool spectrum of the rat depends on the load of dietary cholesterol. We have observed significant decreases in cholic acid and compensating increases in chenodeoxycholic acid, when diets supplemented with cholesterol are fed (8). Hormonal changes also alter the pool spectra (4).

In Figs. 2 and 3 we have plotted bile

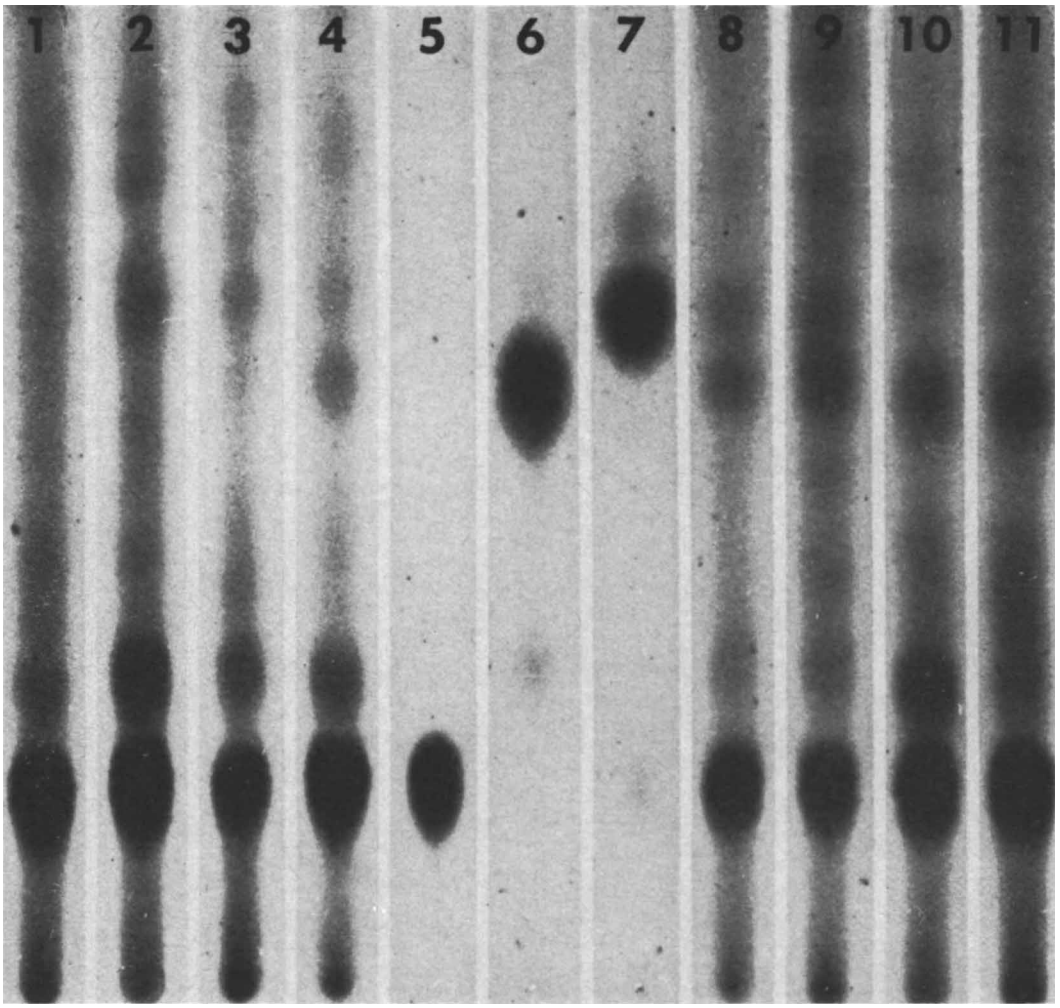

FIG. 1. Ascending thin-layer chromatogram of bile acid pools of various rodents: channels 1 and 2, mouse; 3 and 4, gerbil; 5, standard cholic acid; 6, standard chenodeoxycholic acid; 7, standard deoxycholic acid; 8 and 9, hamster; 10 and 11, rat. Solvent system: isooctane:ethyl acetate: acetic acid, 10:10:3 (v/v). Spray: 20% phosphomolybdic acid in ethanol.

acid-^{14}C fecal excretion data from which the half-lives of the cholic and chenodeoxycholic pools in the various rodents are calculated. The time in days is plotted on the ordinate against $-\log(1 - u^t/u^{max})$ on the abscissa. The u^t = the fecal bile acid-24-^{14}C excretion (cpm) up to and including a given day; u^{max} = the total bile acid-^{14}C recovered in the tissues and feces of a given animal. The straight lines obtained show that bile acid excretion is governed by first order kinetics in each of the species. The half-life of the bile acid pool can be read from the plot at the point where $u^t/u^{max} = 0.5$. The dotted vertical line intersects the rate line for each plot at the half-life of the bile acid pool. The half-lives (days) of the cholic acid pools were: rat = 3.5 ± 0.4, mouse = 5.0 ± 0.3, gerbil = 2.3 ± 0.2, hamster = 1.0 ± 0.2. For the chenodeoxycholic acid pools, the half-lives were: rat = 2.0 ± 0.2, mouse = 2.5 ± 0.5, gerbil = 1.3 ± 0.1, hamster = 1.8 ± 0.3.

It is important to note that in each species the half-life of the chenodeoxycholic acid pool differs from that of the cholic acid pool.

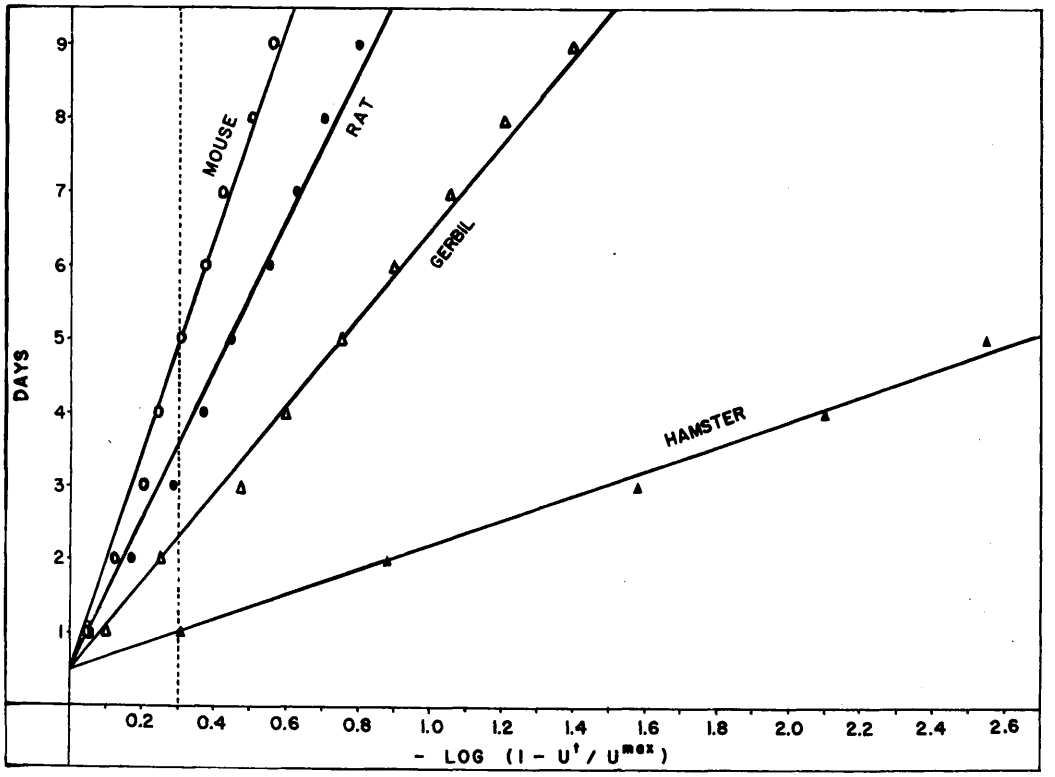

FIG. 2. Rate of elimination of cholic acid from rodent bile acid pools: The vertical dotted line cuts the curves at the cholic acid half-life.

This finding was not unexpected since cholic and chenodeoxycholic acid are metabolized via different pathways by intestinal bacteria and the products of this metabolism are most likely absorbed at different rates. In those animals which have relatively large chenodeoxycholic acid pools, the differing half-lives could be of importance in determining bile acid excretion. This could have significance since the ratio of cholic to chenodeoxycholic acid changes under different hormonal and dietary circumstances (4, 8).

The bile acid pool half-life does not correlate with the relative rate of accumulation of tissue cholesterol in animals fed cholesterol-supplemented diets. For example, although the bile acid half-life in the hamster and gerbil is much shorter than in the rat and mouse, the former animals accumulate large amounts of serum and liver cholesterol, while the latter do not (9, 10).

Before we dismiss the possibility of a correlation between bile acid synthesis and proneness to accumulate tissue cholesterol, we must examine the sizes of the bile acid pools in the various animals. This is necessary since both the bile acid half-life and turnover time must be known to calculate the rate of conversion of liver cholesterol to bile acids (bile acid synthesis). Pool sizes are shown in Table III. All of the pools were calculated per animal, but average animal

TABLE III. Bile Acid Pool Sizes in Various Rodents.

Species	Wt. (g)	Pool size (mg/animal) Cholic acid	Chenodeoxycholic	Total
Rat	260	21.12 ± 2.82	6.06 ± 1.83	27.18
Mouse	23	5.62 ± 1.09	Trace	5.62
Hamster	100	1.94 ± 0.53	0.33 ± 0.13	2.27
Gerbil	75	7.12 ± 1.26	Trace	7.12

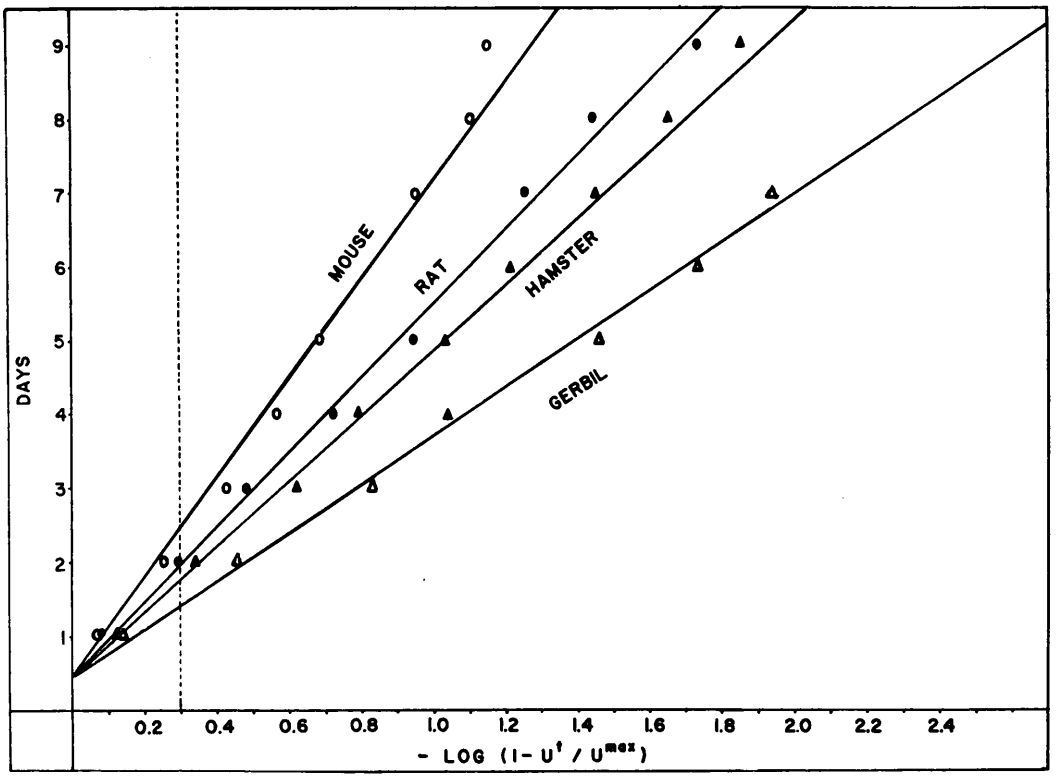

FIG. 3. Rate of elimination of chenodeoxycholic acid from rodent bile acid pools: The vertical dotted line cuts the curves at the half-life.

weights are also given so that a comparison can be made per unit weight.

Since in one-hald-life half of the bile acid pool is turned over, we can calculate the turnover time, $\overline{T}$, and the daily synthesis rate of bile acids in each of the rodents. The synthesis rates are shown in Table IV. On a mg/day/animal basis, the rat synthesized and excreted by far the largest amount of total bile acids, followed by the gerbil, hamster, and mouse in that order. These rates are directly proportional to the absolute amounts of cholesterol metabolized to bile acids and excreted per day. If an equal amount of cholesterol were deposited by each species, the absolute rate of mobilization of the deposited sterol via the bile acid pathway would be in the above mentioned order. It is questionable, however, that the rate of bile acid synthesis would correlate with the propensity of a species to accumulate blood and liver cholesterol unless bile acid synthesis were a prime determining factor in the process.

An analysis of the processes involved in tissue cholesterol accumulation shows that the situation is complex, and that several factors determine the rate of accumulation. These include the rates of cholesterol absorption, deposition and mobilization, and the size of the sterol pool. Since the sterol pool sizes in these animals are approximately proportional to their body weights, a comparison of the relative rates of cholesterol accumulation in these rodents (taking into consideration their daily exogenous cholesterol consumption) with the rates of bile acid synthesis, on a mg/day/100 g of animal basis, should give some idea of the importance of bile acid excretion rates in determining the rate of blood and liver sterol accumulation in the different species. The rate of synthesis in mg/day/100 g of animal (Table IV) was about the same in the rat and gerbil, and

somewhat faster in the mouse. The hamster had about half the rate of bile acid synthesis as the rat. If we compare cholesterol accumulation in these rodents (9, 10), maintained on cholesterol-supplemented diets which supplied 34, 33.5, 87 and 30 mg/day/100 g of animal, respectively, we find that the mouse and rat accumulated very little blood and liver cholesterol, while the gerbil accumulated considerable concentrations; and the hamster tremendous amounts. The mouse obviously is very refractory to cholesterol accumulation, probably because a combination of factors—including a relatively fast rate of mobilization via the bile acid pathway—operates to prevent sterol accumulation. It is evident that the gerbil either absorbs cholesterol and/or deposits it in its pool more readily than the rat. While the hamster had the slowest rate of conversion of sterol to bile acids of any of the rodents studied, a combination of factors seems to be responsible for the high rate of accumulation.

It is obviously difficult to draw conclusions as to the importance of cholesterol mobilization via the bile acid pathway in preventing cholesterol accumulation in these rodents without further studies on the relative rates of absorption and deposition of cholesterol in the blood and liver pools. In addition studies on the effects of accumulation of blood and liver cholesterol on bile acid synthesis in various species are needed, since there may be important changes in the half-life of the bile acid pools under these condtions (8).

Summary. A comparative study of bile acid metabolism was made in the rat, mouse, hamster, and gerbil. Bile acid elimination was mainly via fecal excretion; less than 7% was excreted in the urine. Little or no bile acid side-chain oxidation took place in any of the species. A study of bile acid distribution in the rodent tissue showed that from 80 to 90% was present in the small intestine plus gall bladder. The cecum was another site which contained considerable quantities of bile acids. The chief pool bile acid in each case was cholic acid. Chenodeoxycholic acid was present in significant amounts in rat and hamster, and in trace amounts in mouse and

TABLE IV. Bile Acid Pool Half-Lives, Turnover Times, and Synthesis Rates in Various Rodents.

Species	Food consumption (g/100 g of animal)	Half-life (days)		Turnover time[a] (days)		Synthesis rate (mg/day/animal)			Synthesis rate (mg/day/100 g of animal)		
		Cholic acid	Chenodeoxycholic	Cholic acid	Chenodeoxycholic	Cholic acid	Chenodeoxycholic	Total	Cholic acid	Chenodeoxycholic	Total
Rat	4.6	3.5	2.0	5.1	2.9	2.09	1.05	3.14	0.80	0.40	1.20
Mouse	17.4	5.0	2.5	7.2	3.6	0.39	—	0.39	1.69	—	1.69
Hamster	6.0	1.0	1.8	1.4	2.6	0.67	0.06	0.73	0.67	0.06	0.73
Gerbil	6.7	2.3	1.3	3.3	1.9	1.07	—	1.07	1.43	—	1.43

[a] Turnover time = half-life/(ln 2).

gerbil bile. Cholic acid half-lives (days) were: rat, 3.5; mouse, 5.0; gerbil, 2.3; and hamster, 1.0. Chenodeoxycholic half-lives (days) were: rat, 2.0; mouse, 2.5; gerbil, 1.3; and hamster, 1.8. The rat had the largest bile acid pool (27.2 mg); the mouse (5.6 mg) and gerbil (7.12 mg) intermediate amounts; and the hamster the smallest pool (2.3 mg). The relationship between bile acid synthesis rates and the rate of accumulation of tissue cholesterol after feeding cholesterol-supplemented diets was discussed. Positive correlations were noted but further studies are necessary to define the relative importance of this factor in maintaining cholesterol homeostasis in the various rodents.

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